

DEVELOPMENT OF CHITOSAN AND SODIUM ALGINATE COMPOSITE COATING ON 316L STAINLESS STEEL BY ELECTROCHEMICAL METHOD

A Thesis submitted in partial fulfilment of the requirements for the degree

of

Master of Technology

In

Biomedical Engineering

By

CHANDRA PRAKASH

212BM1354



Department of Biotechnology & Medical Engineering

National Institute of Technology

Rourkela-769008, Orissa, India

DEVELOPMENT OF CHITOSAN AND SODIUM ALGINATE COMPOSITE COATING ON 316L STAINLESS STEEL BY ELECTROCHEMICAL METHOD

A Thesis submitted in partial fulfilment of the requirements for the degree

of

Master of Technology

In

Biomedical Engineering

By

CHANDRA PRAKASH

212BM1354

Under The guidance of

Dr. Amit Biswas



Department of Biotechnology & Medical Engineering

National Institute of Technology

Rourkela-769008, Orissa, India



National Institute of Technology

Rourkela

CERTIFICATE

This is to certify that the thesis entitled, “**Development of chitosan and sodium alginate composite coating on 316L stainless steel by electrochemical method**” submitted by **Chandra Prakash** is an authentic work carried out by him under my supervision and guidance for the partial fulfillment of requirements for the award of **Master of Technology in Biomedical Engineering at National Institute of Technology Rourkela**. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University / Institute for the award of any Degree or Diploma.

Place: NIT Rourkela
Date: 25 May,2014

Dr. Amit Biswas
Asst. Professor
Dept. of Biotechnology & Medical Engg.
National Institute of Technology Rourkela

ACKNOWLEDGEMENT

Successful completion of this project is the outcome of consistent guidance and assistance from many people, faculty and friends and I am extremely fortunate to have got these all along the completion of the project.

I owe my profound gratitude and respect to my project guide, **Prof. A. Biswas**, Department of Biotechnology and Medical Engineering, NIT Rourkela for his invaluable academic support and professional guidance, regular encouragement and motivation at various stages of this project.

I place on record my sincere gratitude to **Prof. Krishna Pramanik**, Head of Department, Department of Biotechnology and Medical Engineering, NIT Rourkela for her constant encouragement.

I would like to thank **Ms. Sahely Saha, Ms. Tejinder Kaur, Mr. Dipanshu, Mr. Ravi, Mr. Saikat and Mr. Mohit**, Department of Biotechnology and Medical Engineering, NIT Rourkela, for their regular support, help and motivation.

I would also thank my Institution and my faculty members without whom this project would have been a distant reality. I also extend my thanks to my family, friends, and well-wishers.

Place: NIT Rourkela
Date: 25 May,2014

Chandra Prakash
212BM1354
Biotechnology and Medical
Engineering
National Institute of Technology
Rourkela-769 008, Odisha (India)

Table of contents

Contents	Page
List of tables	vi
Abbreviations	vii
CHAPTER 1:	1
INTRODUCTION	1
1.1 Introduction	2
1.2 Objectives and scope of the present study.....	4
1.3 Overview of the thesis	4
CHAPTER 2:	5
LITERATURE REVIEW	5
2.1 Stainless steel	6
2.2 Different polymer coatings	6
2.3 Electrophoretic deposition.....	9
2.4 Mechanism of electrophoretic deposition	9
CHAPTER 3:	11
MATERIALS AND METHODS	11
3.1 Materials Required	12
3.2 Sample Preparation.....	13
3.3 Solution preparation	13
3.4 Experimental procedure for electrophoretic deposition	15
3.5 Characterization techniques	15
3.5.1 Optical microscopy	15
3.5.2 X-ray diffraction	16
3.5.3 Fourier transform infrared spectroscopy (FTIR)	17
3.5.4 Wettability	18
CHAPTER 4:	19
RESULTS AND DISCUSSION	19
4.1 Optical microscopy.....	20
4.2 XRD analysis.....	22
4.3 FTIR Analysis.....	24

4.4 Wettability Analysis.....	25
CHAPTER 5:	27
CONCLUSION	27
CHAPTER 6:	29
REFERENCES	29

List of figures

Figure	Page
Figure 1: Experimental set up of electrophoretic deposition process	10
Figure 2: Optical microscope	16
Figure 3: XRD equipment (Panalytical) with inbuilt software for data collector	17
Figure 4: IRPrestige-21 machine used for FTIR	18
Figure 5: Shows the optical microscopic images of (a) pure chitosan, (b) 0.25% sodium alginate, (c) 0.50% sodium alginate and (d) 0.75% sodium alginate in chitosan solution, coated on 316L stainless steel	21
Figure 6: Shows the optical microscopic images of the chitosan- sodium alginate coating deposited (0.50 wt% sodium alginate) for (a) 5 min, (b) 10 min, (c) 15 min and (d) 20 min	22
Figure 7: XRD profile of stainless steel sample (a) before coating, (b) with chitosan coating and (c) with chitosan- sodium alginate coating (0.50% sodium alginate, 15 min)	23
Figure 8: FTIR of (a) pure sodium alginate, (b) pure chitosan and (c) chitosan-sodium alginate composite coating	24
Figure 9: Contact angle of (a) poshished stainless steel, (b) chitosan coated stainless steel and (c) chitosan-alginate composite coated stainless steel (0.50 % sodium alginate, 15 min)	26

List of tables

Table	Page
Table 1: The bath compositions and deposition conditions for electrophoretic deposition process	14
Table 2: Contact angle for bare surface and coated surface	25

Abbreviations

μm	Micro meter
EPD	Electrophoretic Deposition
HA	Hydroxyapatite
cm	Centimeter
ft.	Foot
ml	Milliliter
gm	Gram
ltr	Litre
min	Minute
V	Volts
rpm	Revolutions per minute
wt%	Weight percent
%	Percent
°	Degree

Abstract

Stainless steel has been used as material for orthopedic and orthodontic implants due to their low cost, good formability and proper mechanical properties. However, stainless steel is susceptible to pitting corrosion in body fluids and have poor wear resistance which limits its applications. Surface coating with certain ceramic and polymer composites are done to improve the biocompatibility of stainless steel. Chitosan is the cationic regular biopolymer having a unique structure, multidimensional properties, highly sophisticated function and extensive variety of requisition in biomedical field. Chitosan is a promising biomaterial for medical implants due to its biocompatibility, biodegradability and nontoxic property alongside its antimicrobial action and low immunogenicity. A chitosan and chitosan-sodium alginate composite layer has been electrophoretically deposited on 316L stainless steel. The solution comprises of distilled water and acetic acid. The deposition of chitosan and sodium alginate was carried out for 5, 10, 15 and 20 min at constant power supply of 20V on the cathode. The morphological analysis using optical microscopy, phase purity analysis using X-ray diffraction (XRD), molecular structure analysis using Fourier transform infrared spectroscopy (FTIR) and wettability by sessile drop method was done to characterize the samples. FTIR indicated the existence of bond between protonated carboxylate bonds and partially protonated amine group which will enhance the protein adhesion on the coated surface.

Keywords: *biocompatibility, chitosan, stainless steel, sodium alginate, electrophoretic deposition*

CHAPTER 1:

INTRODUCTION

1.1 Introduction

A wide variety of materials are utilized for medicinal purposes. The metallic biomaterials are specially used where load bearing structures are needed, for example, in dentistry and orthopedics. The main advantages of utilizing metallic biomaterials is that a both basic and complex shapes implants can be fabricated with the help of well-known techniques like forging, casting and machining. Biomaterials are made from different metals like stainless steel, tantalum, titanium and nickel titanium alloys. Biomaterials are made from different metals like stainless steel, tantalum, titanium and nickel titanium alloys. Stainless steel (316L SS) is broadly used for fracture repair devices and joint replacement parts. In comparison to other metallic implants stainless steel has low cost, proper mechanical properties and good formability thus makes it a suitable metallic implant material [1]. However, the susceptibility of stainless steel to pitting corrosion in body fluids, biocompatibility, poor wear resistance and the low surface hardness have limited their applications. So, make the suitable use of implant a suitable surface modification of the material required [2]. Through the coating techniques, the properties of the surface of the material can be altered, without influencing the properties of the whole implant.

A number of coating techniques have been used to coat the metallic implants, which includes plasma spraying, anodic oxidation, sol-gel process, biomimetic coating, sputtering and electrochemical treatment [3]. Electrophoretic deposition (EPD) is one of the most effective methods available for coating of metallic implants due to its low cost and simple experimental setup. The main advantages of EPD over other coating techniques are low deposition time, simple apparatus, high coating uniformity with control over thickness (0.3- 100 μm) and its capacity to coat complex 3D shapes [4]. The mechanism of the electrophoretic deposition is directly related with the pH variation which is caused due to electrochemical decomposition of water.

Biocompatible and biodegradable polymers particularly from natural origins have been utilized as EPD coating materials. The polymer molecules are highly charged.

An applied electrical field drives these molecules to move and to condense on the substrate [5]. These biodegradable polymers not only act as adhesive between the interfacing of chitosan and the surface of medical implants, but also offers to control the in-situ release of products by which better bone growth might be foreseen. Several functional groups for example, amine, hydroxyl and carboxyl groups can enhance the adsorption of proteins and cell adhesion, giving a suitable environment on the surface of the implant for bone growth [6]. Most important biodegradable polymers used in biomedical applications are poly ethylene glycol, poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly (ε-caprolactone) (PCL) , poly (3-hydroxybutyrate) (PHB), copolymers of polyglycolide, chitosan, alginate and soy protein [7].

Chitosan is a cationic polysaccharide and one of the most useful natural biopolymers for tissue building, bio mimetic coatings and in delivery of drugs. It has attracted a lot of attention in wide variety of requisitions running from skin, bone, vascular grafts and cartilage to substrates for cell culture due to its special properties such as biodegradability, biocompatibility, bio-functionality and non-toxicity. Past studies have indicated the cationic nature of the chitosan and it shows that chitosan can be electrophoretically deposited [8]. Alginate is a natural anionic polysaccharide determined from green algae, and has been highly used for drug delivery, biosensing, tissue engineering and other biomedical applications due to its cost-effectiveness, biocompatibility and low toxicity. Chitosan and sodium alginate are natural polymers, which are nontoxic, biodegradable, biocompatible and pH sensitive, while also being polyelectrolytes that have amino and carboxyl groups. Thus, they are widely used in the formation of microparticles through electrostatic attraction [9].

In this study a layer of chitosan and composite of chitosan and sodium alginate was deposited by electrophoretic deposition method on stainless steel substrate to improve the bioactivity of the surface and characterization of the coating was done to see the compositional effect on the coating property.

1.2 Objectives and scope of the present study

The aim of the present work is to deposit chitosan and its composite on 316L stainless steel by electrophoretic method. The objectives could be listed like underneath:

- ❖ To develop a chitosan and sodium alginate composite coating on 316L stainless steel by electrophoretic method and compare it with pure chitosan coating
- ❖ To study the effect of various composition of sodium alginate and chitosan on the formation of composite layer on 316L stainless steel.
- ❖ To study the effect of deposition time on the formation of composite layer
- ❖ Characterization of the coated samples for morphological study (Optical microscopy), phase purity (XRD) and molecular structure (FTIR) and wettability.

1.3 Overview of the thesis

The overview of the rest of the thesis is as follows. In chapter 2, the method of electrophoretic deposition, its mechanism, applications, parameters for enhancing coating, surface morphology changes with parameters and short survey of literatures related to electrophoretic deposition are given. In chapter 3, a brief experimental setup and different characterization techniques are given. In chapter 4, determination of the coated samples in terms of surface morphologies, phase structural change of the coated material are presented. An outline of the principle discoveries alongside conclusions is presented in chapter 5. Chapter 6 contains the references.

CHAPTER 2:

LITERATURE

REVIEW

2.1 Stainless steel

Stainless steel has been used as material for orthopedic and orthodontic implants due to its low cost, good formability and appropriate mechanical properties. But it has poor corrosion resistance and non-biocompatible [2].

The load bearing implants are subjected to different stresses and needs to be strong enough to bear the stresses which are incurred upon them by the body. The implants ought to have high resistance to corrosion, good mechanical properties, have good fatigue life, and experience no wear and tear while functioning. Normally, metals and metallic alloys have been utilized as implant materials because they satisfy a lot of these requirements. 316L stainless steel, has been progressively utilized as implant material, as a result of their similarities to human bone in terms of hardness, young's modulus of elasticity and other mechanical properties [10]. 316L stainless steel is recognized as one of the appealing metallic materials for biomedical applications because of its mechanical properties, biocompatibility and corrosion resistance. It has emerged as a popular metal for implantation as acetabula cup (one half portion of a hip joint) applications [11]. However, the susceptibility of stainless steel to pitting corrosion in body fluids, non-biocompatibility, poor wear resistance and the low surface hardness have limited their applications. So, to put the implant into a specific use, a suitable surface modification of the material is required [2]. Through the coating techniques, the surface properties of stainless steel can be altered without affecting the properties of bulk material.

2.2 Different polymer coatings

There are number of biopolymers that can be used as coating material on metallic implants to improve the bio compatibility of the implant. The biodegradable polymers mostly used in biomedical applications include:

- ❖ **Chitosan** - Chitosan is a cationic polysaccharide and one of the most useful natural biopolymers for tissue building, bio mimetic coatings and in drug delivery. It has attracted a lot of attention in wide variety of applications

running from skin, bone, vascular grafts and cartilage to substrates for cell culture, due to its special properties such as biodegradability, biocompatibility, bio-functionality and non-toxicity. Past studies have shown that chitosan and chitosan mediated coatings have been developed for the immobilization of nucleic acids, proteins, and virus particles in chitosan and proved that the surface characteristics of the metallic implants can be positively influenced by the chitosan coating [8]. It has also been studied that chitosan- bio active glass composite layer have been deposited on titanium implants by electrophoretic deposition. The coating layer increases the particle size and porosity of the surface layer and corrosion resistance shifted towards more noble values [12]. In recent times polymer ceramic coatings have also been deployed on stainless steel substrate. The chitosan-HA coating has been deposited on the stainless steel by electrophoretic deposition, which enhance the corrosion resistance of the stainless steel [13]. A novel chitosan-titania nanoparticle composite coating on stainless steel have also been deposited through electrophoretic deposition for biomedical purpose which increases the hydrophilicity and corrosion resistance of the implant surface [14].

- ❖ **Alginate** – Alginate is a linear polysaccharide, obtained from green algae, found abundantly in nature. Past studies have indicated that alginate coatings can be deposited by electrophoretic deposition on metallic implants and this coatings have potential applications in the field of tissue engineering and scaffold fabrication [15]. Alginate- bioglass composite coatings deposited by electrophoretic method on stainless steel have been found to be a suitable candidate for applications in orthopedic, dental metallic implants and for bone tissue scaffolds [6]. Calcium- alginate composite coatings have been deposited on titanium implants through dip coating to control the release of the water soluble drugs. The coated surface might be applied as a bioactive, biodegradable layer on the bio-inert surface of the implant, which induces the formation of apatite and actively bond to the surrounding tissue [16].

- ❖ **Gelatin** – Gelatin is a biopolymer mainly used in composite coatings to enhance the biocompatibility, such as cell adhesion and proliferation due to its excellent cellular affinity. Past studies have indicated that gelatin composite coatings can be electrophoretically deposited on the metallic implants and have enormous applications in tissue repair and drug delivery [4]. The bioactive gelatin and calcium phosphate composite coatings have also been coated on titanium by dip coating technique. These coatings are beneficial for mesenchymal stem cells (MSCs) proliferation, osteogenic differentiation and also favorable for bone bonding ability on titanium implants. It has also been suggested that composite coating might have great potential in dental and joint replacement implants [17]. A composite coating of strontium (Sr) doped gelatin and calcium phosphate has been deposited by electrophoretic deposition on titanium implants to increase the corrosion resistance and bioactivity of the implants. This also enhances the in vitro biocompatibility of the titanium implants [18]. It has also been studied that gelatin methacrylate and HA composite coatings deposited on titanium implants by photochemical approach have increased the hydrophilicity of the implant. It is expected that the composite coatings might promote and accelerate the osseointegration for bone regeneration and repair of the titanium implants [19].
- ❖ **Poly ethylene glycol (PEG)** – PEG have high hydrophilicity, non-toxicity and non-immunogenicity, which makes it a suitable coating material for metallic implants. Past studies have shown that PEG composite coatings can be deposited on stainless steel stent by sol-gel method, which enhance the hydrophilicity and thus reduces the activation and adhesion of platelets [20].
- ❖ **Poly lactic acid (PLA)** – Polylactic acid (PLA) based materials have been applied in biomedical applications due to their bioresorbable properties. Past studies have suggested that the PLA can be deposited on stainless steel by dip coating method. The PLA coating deposited by the dip coating assists in drug delivery and tissue regeneration applications [21].

❖ **Silk protein sericin** – Sericin exhibits various biological properties that recognizes it as a potential material in pharmacological, biomedical and biotechnological field. Recent studies have shown that sericin can be deposited on the titanium implants and these sericin immobilized titanium surfaces are potentially useful bioactive coated materials for titanium-based medical implants [22].

2.3 Electrophoretic deposition

Electrophoretic deposition is a conventional technique requiring minimal efforts and low energy. It has advantages like competence to handle complex geometry, basic scale-up with effectively low supplies and great chemical stability. Using this technique a very large number of pure metals, ceramics or composites can be electrodeposited with grain size of less than 100 nm. Materials coated by this technique have industrial applications which include coatings of engine cylinders, high pressure valves, small aircraft microelectronics, aerospace, car accessories, marine, agriculture, nuclear field and medical devices [23].

2.4 Mechanism of electrophoretic deposition

Electrophoretic Deposition is the method by which charged ions in the solution are attracted and coated onto the oppositely charged electrode under the influence of an electric field. Electrophoretic deposition is basically a two-step process. In the first step the charged ions move under the influence of electric field to the oppositely charged electrode. In the second step, the particles stored on the electrode shaping a moderately thick, compact and homogenous films. There are two sorts of electrophoretic deposition techniques on the basis of the electrode on which the coating is done. When the particles are positively charged and the deposition of the layer occurs on the cathode and the methodology is called cathodic electrophoretic process and if the particles are negatively charged the deposition occurs on the anode and the procedure is known as anodic electrophoretic deposition. The process leads to the deposition of the single material and composite layer decided by the powder used

for the preparation of the solvent. Basically the principle of the electrophoretic deposition is the electrophoretic mobility of the charged particle in the solvent under the influence of applied electric field.

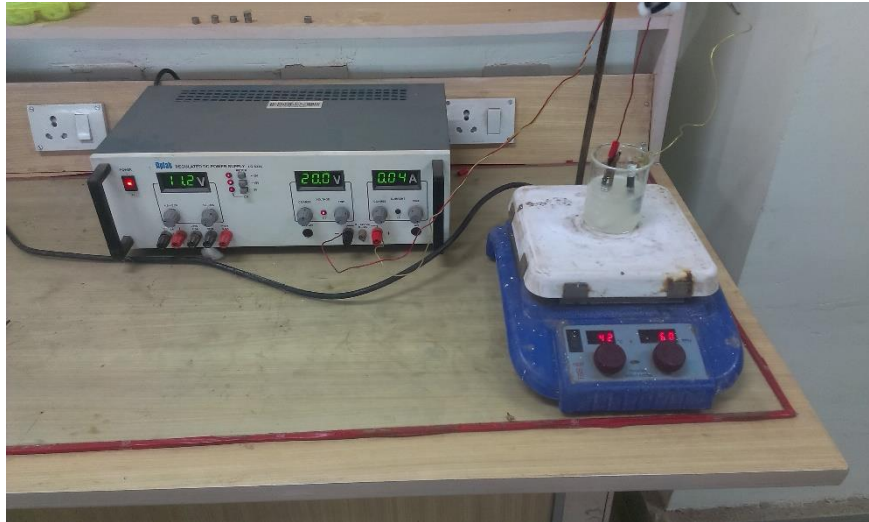


Figure 1: Experimental set up of electrophoretic deposition process

CHAPTER 3:

MATERIALS AND

METHODS

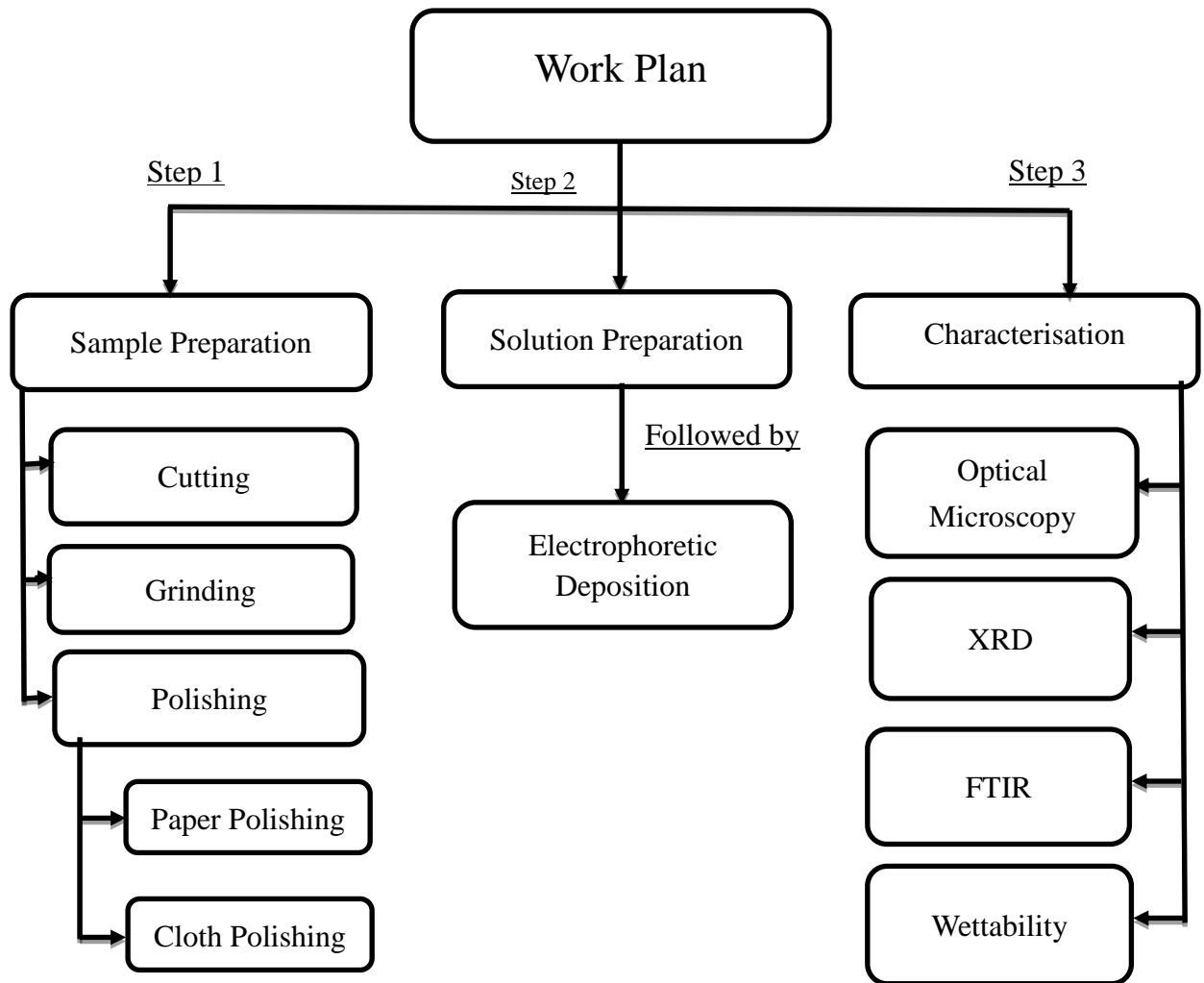
3.1 Materials Required

❖ *For Sample Preparation*

- Stainless steel rod of diameter 1 cm and 1 ft long
- Hexo blade
- Belt grinder
- SiC papers for poslishing of grade 1/0, 2/0, 3/0 and 4/0
- Alumina paste for cloth polishing
- Diamond paste with hifin solution for final finishing
- Distilled water and ethanol for cleaning of samples

❖ *For Solution Preparation*

- Acetic acid
- Distilled water
- Chitosan
- Sodium alginate
- Magnetic Stirrer



3.2 Sample Preparation

Firstly, cutting of stainless samples in the form of rod was done, followed by the standard metallographic techniques to develop the scratch free surface. Polishing was done in two phases which includes paper polishing and cloth polishing. Hifin solution was used to give the final touch on the polished surface.

3.3 Solution preparation

Chitosan was dissolved in the solution of 4 ml acetic acid in 396ml of distilled water. Thereafter, solution was taken in four separate beakers of 100 ml each. Then 0.025 gm, 0.05 gm and 0.075 gm sodium alginate was dissolved in the three beaker, while

fourth one was kept as pure chitosan solution. The pH of the solutions was maintained in the range of 3.3 to 3.6. Then all the solutions were magnetically stirred for 2 hours at room temperature.

Table 1: The bath compositions and deposition conditions for electrophoretic deposition process

Electrolyte	4 ml - Acetic Acid 396 ml – Water
Polymer used	Chitosan - 1gm/ ltr Sodium Alginate in different concentrations
Solution 1 (100 ml)	Chitosan – 0.1 gm Alginate – 0 gm
Solution 2 (100 ml)	Chitosan – 0.1 gm Alginate – 0.025 gm
Solution 3 (100 ml)	Chitosan – 0.1 gm Alginate – 0.050 gm
Solution 4 (100 ml)	Chitosan – 0.1 gm Alginate – 0.075 gm
pH	3.3 – 3.6
Applied Voltage (Volts)	20 V
Deposition time	5 min, 10 min, 15 min, 20 min

3.4 Experimental procedure for electrophoretic deposition

- ❖ Initially electrodes were washed with acetone and then dried at 37⁰ C (room temperature).
- ❖ The deposition was carried out at room temperature in 100 ml beaker with a continuous magnetic stirring of 60 rpm.
- ❖ Graphite rod was used as anode and sample was placed at cathode, as the polymers in use were cationic polymers.
- ❖ The distance between the sample and graphite rod was maintained at 2 cm.
- ❖ The chitosan was deposited at constant voltage of 20V for time periods of 5 min, 10 min, 15 min and 20 min.
- ❖ The chitosan composite was deposited at constant voltage of 20V for time periods of 5 min, 10 min, 15 min and 20 min.

3.5 Characterization techniques

3.5.1 Optical microscopy

Optical microscope (ZEISS) with the facility of image analyser was used for characterization. The reflected light mode was used so that specimen illuminated due to the opacity of metal. The prepared specimen was placed over the horizontal stage with surface perpendicular to the optical axis of the optical microscope and illuminated through the objective lens by light from a lamp or arc source. This light was focused by the condenser lens into a beam which was adjusted approximately parallel to the optical axis of the microscope by a half-silvered mirror. The light then passed through the objective on the specimen and then reflected from the surface of the specimen, back through the objective, the half-silvered mirror, and then to the eyepiece to the observer's eye, or to a camera port or a film plane.

Machine Name – Optical Microscope

Manufacturer – ZEISS (Axiotech)

Software Used – Axovision 4.8



Figure 2: Optical microscope

3.5.2 X-ray diffraction

The identification of phase and crystallinity of the coated material was analysed by X-ray diffraction method. A Cu target was utilized as X-ray source (CuK-radiation). A graphite monochromator was spotted before the relative counter to decrease the background noise in the detector. X-rays diffraction profile was observed in the region of $5^\circ < 2\theta < 70^\circ$ with scan rate of $3^\circ/\text{min}$. The diffraction patterns thus produced were compared to the existing data using a JCPDS file. The phases present were identified by comparing the location of the peaks found in XRD profiles to the reference spectrum.

Machine Name - X'PERT PANalytical X-ray diffractometer

Manufacturer – PANalytical

Software used – X'pert data collector (for data collection)



Figure 3: XRD equipment (Panalytical) with inbuilt software for data collector

3.5.3 Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy was used to follow the conformational changes that occurred during the different stages of solution preparation and the course of electrophoretic deposition. With the help of FTIR we can analyse high spectral resolution, signal to noise ratio, capability to measure a wide range of the spectrum in a short measure of time. In FTIR, a broadband source beam of combination of different frequencies incident on the sample and absorbed beam by the sample was measured. Then the source beam was modified to different combinations of the frequencies different to the used in previous source beam and gives the second data point. This process was repeated several times. Thereafter, all the data points were used as input and computer gave the amount of absorption for each wavelength.

Machine Name – IRPrestige-21

Manufacturer – SHIMADZU



Figure 4: IRPrestige-21 machine used for FTIR

3.5.4 Wettability

Wettability is the surface property of the material which can be analyzed by observing the contact angle between the surface of the material and a liquid medium. The wettability test of polished and coated stainless steel samples has been carried out using distilled water.

Method used – Sessile drop method

Camera model – Powershot 600 D

Company Name - Canon

CHAPTER 4:

RESULTS AND DISCUSSION

4. Results and Discussion

The present work was aimed at detailed investigation of electrophoretic deposition of chitosan and chitosan- sodium alginate composite layer on 316L Stainless steel. Electrophoretic deposition of chitosan-sodium alginate composite was carried out under three different wt% of sodium alginate with respect to chitosan. Mixing of sodium alginate with chitosan lead to the formation of poly electrolyte composite solution. Sodium alginate is anionic in nature and was taken in a very small concentration with respect to chitosan, so that the overall charge of the polyelectrolyte composite remain cationic and deposited on the cathode under the applied electric field. The coated surface was then characterized in detail for morphological alteration, phase purity, molecular structure and hydrophilicity in terms of contact angle by using various characterization techniques. The result so obtained are discussed in details in the following section.

4.1 Optical microscopy

Fig 5 shows the optical microscopic images of the pure chitosan and its composite layer formed with different wt% of sodium alginate on 316L stainless steel by EPD process Fig 5 (a) shows that an uniform layer of chitosan was formed which covered the entire steel surface and with the addition of sodium alginate, the morphology of deposited layer was drastically changed from the pure chitosan coating as observed in Fig 5 (b), (c) and (d). Addition of sodium alginate leads to the formation of tiny globular particles on the surface similar to micro spheres. On close observation of the images, it can be depicted that addition of sodium alginate increased the roughness of the coating due to the formation of the microsphere on the material surface at cathode during the deposition.

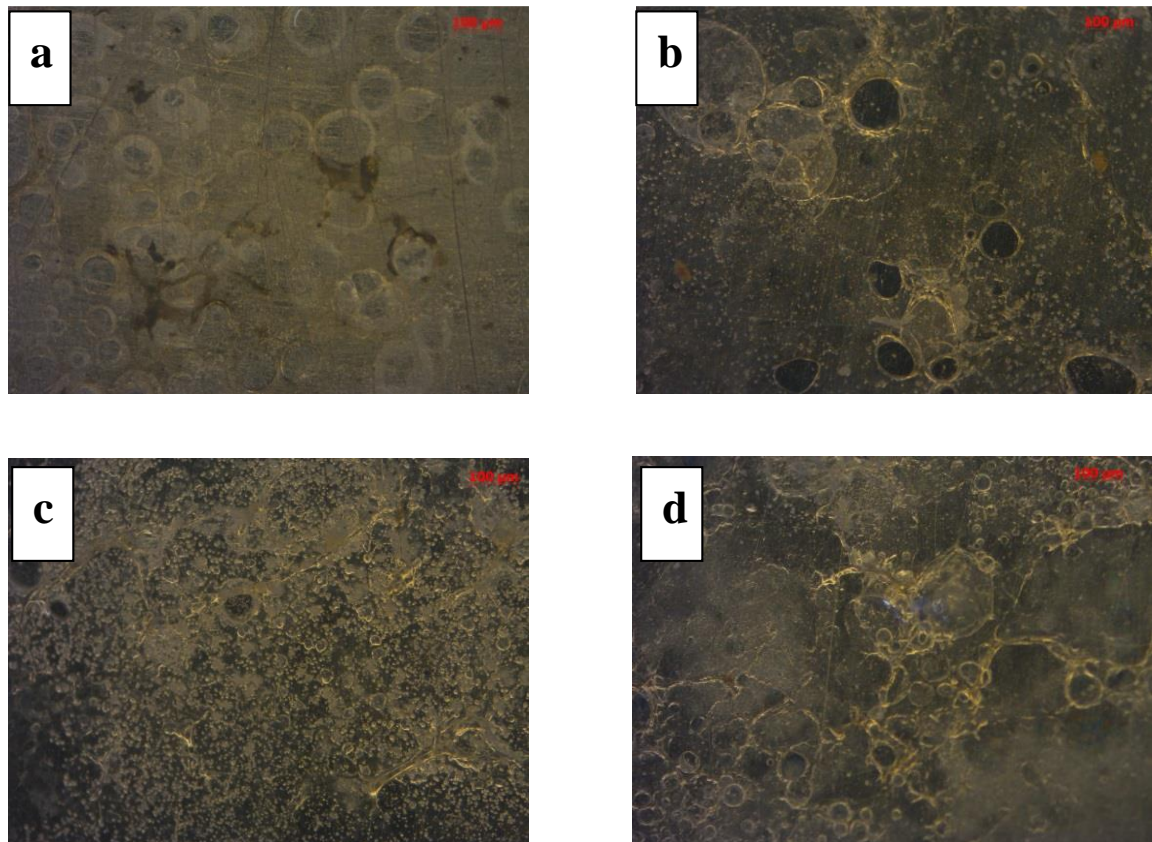


Figure 5: Shows the optical microscopic images of (a) pure chitosan, (b) 0.25% sodium alginate, (c) 0.50% sodium alginate and (d) 0.75% sodium alginate in chitosan solution, coated on 316L stainless steel

Fig 6 shows the effect of deposition time from EPD process on the morphology of the coated layer. It has been observed that deposition time has a strong effect not only on the thickness of the coated layer, it also effects the morphology of the coated surface. It was clearly depicted that the deposition was increased linearly during the initial time of deposition. However, with the increase in deposition time, the deposition rate decreased and attains a saturation at very high deposition time [23].

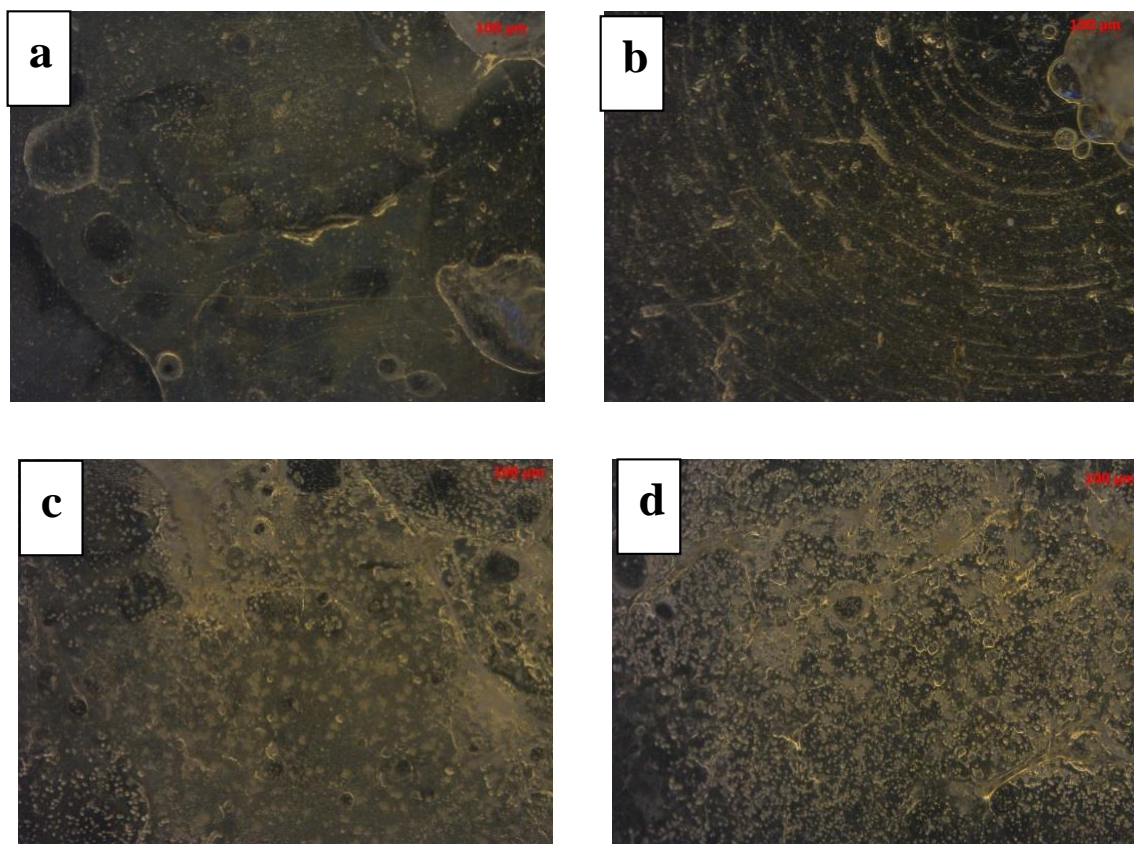


Figure 6: Shows the optical microscopic images of the chitosan- sodium alginate coating deposited (0.50 wt% sodium alginate) for (a) 5 min, (b) 10 min, (c) 15 min and (d) 20 min

4.2 XRD analysis

Fig 7 shows the X-ray diffraction profile of the polished stainless steel, chitosan coated stainless steel and the chitosan-sodium alginate composite coated stainless steel sample. Fig 7 (a) represents the characteristic XRD profile of polished stainless steel sample with peaks of austenite stainless steel at around 43.6° and 50.73° . Fig 7 (b) confirms the presence of chitosan on the coated layer having the characteristic peak of chitosan at 20° indicating the amorphous nature of the chitosan. Fig 7 (c) shows the XRD profile of chitosan-sodium alginate composite coating. A small peak of alginate was found at 34° along with chitosan peak at 20° . From the XRD profile of

(b) and (c) it can be concluded that chitosan and chitosan-sodium alginate are successfully coated on 316L stainless steel and are amorphous in nature. Moreover being an ionic polymer sodium alginate is possible to be deposited on 316L stainless steel by EPD along with chitosan on the cathode from the polyelectrolyte composite solution.

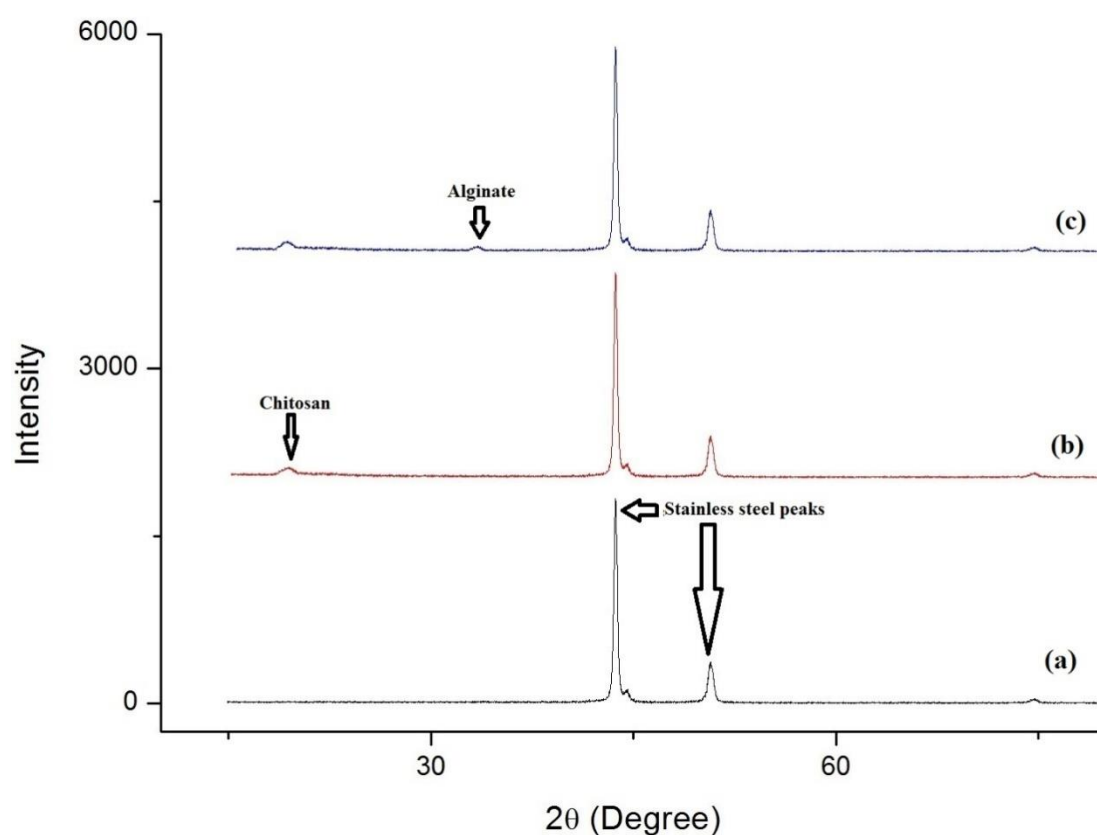


Figure 7: XRD profile of stainless steel sample (a) before coating, (b) with chitosan coating and (c) with chitosan- sodium alginate coating (0.50% sodium alginate, 15 min)

4.3 FTIR Analysis

Sodium alginate (fig 8, plot (a)) shows two different vibrations on the infrared spectrum due to the presence of carboxylate group, a symmetric stretch at 1414 cm^{-1} and an antisymmetric stretch at 1610 cm^{-1} . Peak at 2926 cm^{-1} can be characterized as C-H bond peak. In plot (b) of fig 8, chitosan shows distinct amide I and amide II band at 1661 cm^{-1} and 1560 cm^{-1} respectively. The saturated area between 1000 cm^{-1} and 1150 cm^{-1} is due to the presence of three distinct vibrational modes of C-OC, C-OH and C-C ring vibrations. The broad peak in the range of $3200\text{--}3500\text{ cm}^{-1}$ can be characterized as N-H stretching and O-H stretching vibrations.

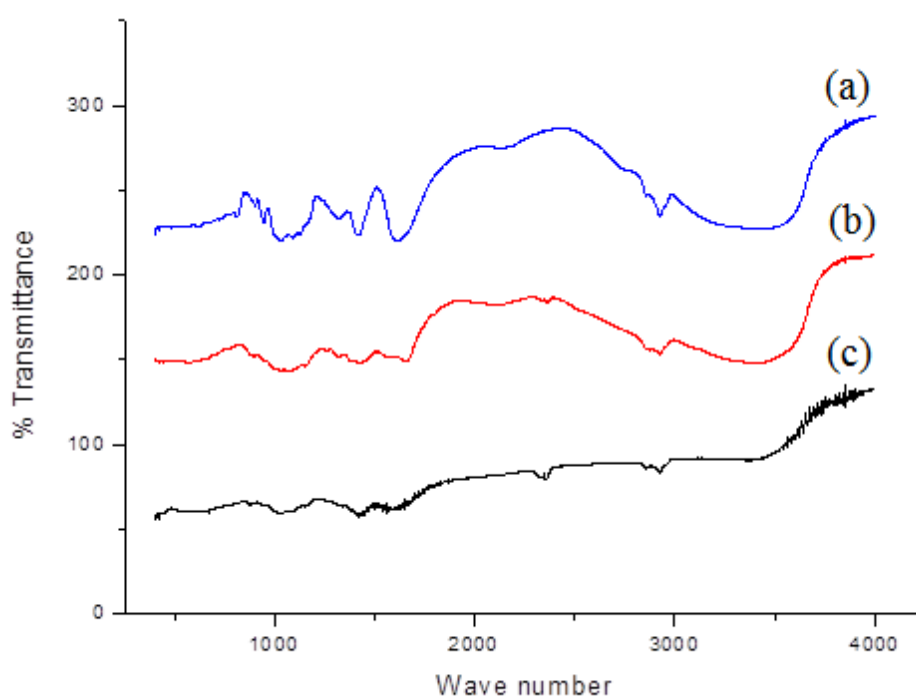


Figure 8: FTIR of (a) pure sodium alginate, (b) pure chitosan and (c) chitosan-sodium alginate composite coating

In the plot (fig 8, plot (c)) of the composite coating of chitosan and sodium alginate a broad peak around 1500-1700 cm^{-1} was found, might be due to merging of several bonds like protonated carboxylate group and partially protonated amine groups. According to Gwen et. al protonation of carboxylate group caused a new band to appear around 1690 cm^{-1} and partial protonation of the amine group of chitosan causes the appearance of one new band (around 1525 cm^{-1}) due to one of the $-\text{NH}_3$ vibrational modes [ref]. The vibration in the region around 1700 cm^{-1} is due to the carbonyl group. C-H bond Peak can be seen around 3000 cm^{-1} . The blending of chitosan and sodium alginate is very good, as low peak was found around 2925 cm^{-1} , which represent that the O-H groups gets reduced after mixing of the polymers. The protonated carboxylate group will increase the protein adhesion of the coating and thus can depicted that the composite coating can improve the bio activity of the 316L stainless steel.

4.4 Wettability Analysis

Wettability is the surface property of the material which can be analyzed by observing the contact angle between the surface of the material and a liquid medium. The wettability test of polished and coated stainless steel samples have been carried out using distilled water and tabulated in the table 2.

Table 2: Contact angle for bare surface and coated surface

Sample	Contact Angle Observed
Polished stainless steel sample	60.03 ⁰
Chitosan coated sample	58.34 ⁰
Chitosan- alginate coated sample	55.94 ⁰

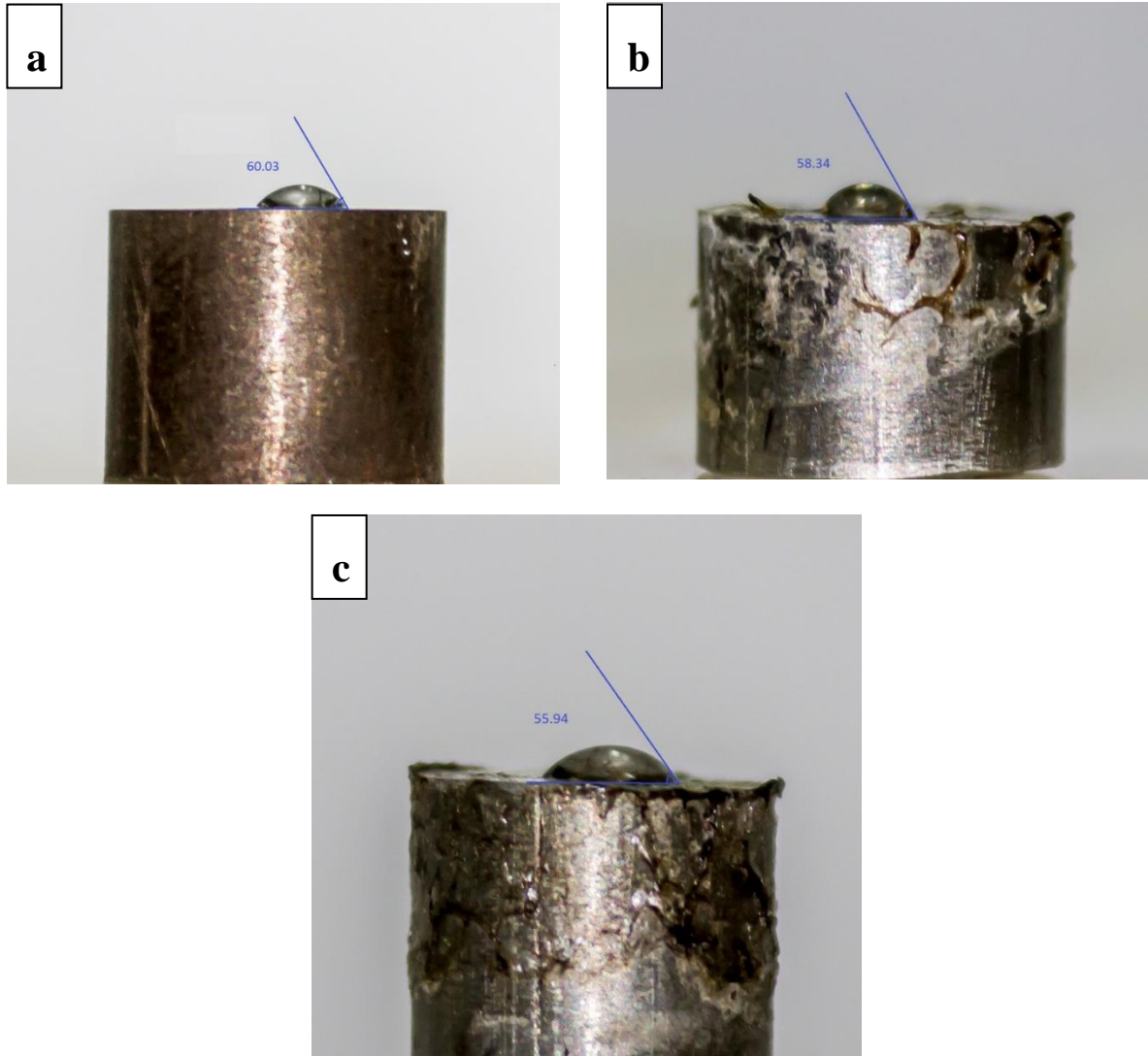


Figure 9: Contact angle of (a) polished stainless steel, (b) chitosan coated stainless steel and (c) chitosan-alginate composite coated stainless steel (0.50 % sodium alginate, 15 min)

From Table 2, it can be observed that the contact angle obtained for a polished stainless steel sample was found to be 60.03° , the contact angles of coated samples were found to decline. This implies that the chitosan-sodium alginate composite coated samples were more wettable/hydrophilic than the pure sample. Thus, increased wettability of the surface will bind the cells more easily when implanted inside the body.

CHAPTER 5:

CONCLUSION

5. Conclusion

It can be concluded that chitosan and sodium alginate composite layer was successfully deposited on 316L stainless steel from polyelectrolyte composite solution by using electrophoretic method.

- ❖ The surface property of the implant require for better bio activity was increased, which was depicted from the result of optical microscopy and wettability.
- ❖ Increased concentration of sodium alginate with respect to chitosan and deposition time enhanced the roughness of the coated layer as observed from the optical microscope.
- ❖ FTIR confirms the bond the protonation of carboxylate bonds and partially protonated amine group which will increases the protein adhesion on the coated surface.

CHAPTER 6:

REFERENCES

6. References

- [1] Liu J, Dong H, Buhagiar J, Song CF, "Effect of low-temperature plasma carbonitriding on the fretting behaviour of 316LVM medical grade austenitic stainless steels," *Wear*, vol. 271, pp. 1490-1496, 2011.
- [2] T. Fu, C.S. Wen, J.Lu, Y.M. Zhou, "Sol-gel derived TiO₂ coating on plasma nitrided 316L stainless steel," *Vacuum*, vol. 86, pp. 1402-1407, 2012.
- [3] S.H. Lee, H.W. Kim, E.J. Lee, "Hydroxyapatite-TiO₂ hybrid coating on Ti implants," *Journal of biomaterials applications*, vol. 20, pp. 195-208, 2006.
- [4] Kapil D. Patel, Rajendra K. Singh, Eun-Jung Lee, Cheol-Min Han, "Tailoring solubility and drug release from electrophoretic deposited chitosan–gelatin films on titanium," *Surface and Coatings Technology*, 2013.
- [5] T. Yoshioka, A. Chavez-Valdez, J.A. Roether, "AC electrophoretic deposition of organic–inorganic composite coatings," *Journal of colloid and interface science*, vol. 392, pp. 167-171, 2013.
- [6] Qiang Chen, Luis Cordero-Arias, Judith A. Roether, Sandra Cabanas-Polo, "Alginate/Bioglass® composite coatings on stainless steel deposited by direct current and alternating current electrophoretic deposition," *Surface and Coatings Technology*, vol. 233, pp. 49-56, 2013.
- [7] Vijay Kumar Malesu, Debasish Sahoo and P.L.Nayak, "Chitosan–sodium alginate nanocomposites blended with Cloisite 30b as a novel drug delivery system for anticancer Drug curcumin," 2011.
- [8] F. Gebhardt, S. Seuss, M.C. Turhan, H. Hornberger, "Characterization of electrophoretic chitosan coatings on stainless steel," *Materials Letters*, vol. 66, pp. 302-304, 2012.
- [9] A.W.G. Nijhuis, S.C.G. Leeuwenburgh, J.A. Jansen, "Wet-Chemical Deposition of Functional Coatings for Bone Implantology," *Macromolecular bioscience*, vol. 10, pp. 1316-1329, 2010.
- [10] T. M. Sridhar, U. K., "Preparation and characterisation of electrophoretically deposited hydroxyapatite coatings on type 316L stainless steel," *Corrosion Science*, vol. 45, pp. 237-252, 2003.
- [11] A Balamurugan., B. G., "Electrochemical and structural characterisation of zirconia reinforced hydroxyapatite bioceramic sol–gel coatings on surgical grade 316L SS for biomedical applications," *Ceramics international*, vol. 33, pp. 605-614, 2007.
- [12] M. Mehdipour and A. Afshar, "A study of the electrophoretic deposition of

- bioactive glass–chitosan composite coating," *Ceramics international*, vol. 38, pp. 471-476, 2012.
- [13] X. Pang and I. Zhitomirsky, "Electrophoretic deposition of composite hydroxyapatite-chitosan coatings," *Materials Characterization*, vol. 58, pp. 339-348, 2007.
- [14] L. Cordero-Arias, S. Cabanas-Polo, Haoxiang Gao, J. Gilabert,, "Electrophoretic deposition of nanostructured TiO₂/alginate and TiO₂-bioactive glass/alginate composite coatings on stainless steel," *Advances in Applied Ceramics*, 2013.
- [15] Zhiliang Wanga, Xueqin Zhang, Juming Gub, Haitao Yang, "Electrodeposition of alginate/chitosan layer-by-layer composite coatings on titanium substrates," *Carbohydrate Polymers*, vol. 103, pp. 38-45, 2014.
- [16] Junwu Xiao, Yingchun Zhu, Yanyan Liu, Yi Zeng, "A composite coating of calcium alginate and gelatin particles on Ti6Al4V implant for the delivery of water soluble drug," *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, vol. 89, pp. 543-550, 2009.
- [17] Zhong-Ming Huang, Yi-Ying Qi, Shao-Hua Du, Gang Feng, "Promotion of osteogenic differentiation of stem cells and increase of bone-bonding ability in vivo using urease-treated titanium coated with calcium phosphate and gelatin," *Science and Technology of Advanced Materials*, vol. 14, p. 055001, 2013.
- [18] Yong Huanga, Yajing Yan, Xiaofeng Pang., "Bioactivity and corrosion properties of gelatin-containing and strontium-doped calcium phosphate composite coating," *Applied Surface Science*, vol. 282, pp. 583-589, 2013.
- [19] Guoxin Tan, Lei Zhou, Chengyun Ningb, Ying Tan, "Biomimetically-mineralized composite coatings on titanium functionalized with gelatin methacrylate hydrogels," *Applied Surface Science*, vol. 279, pp. 293-299, 2013.
- [20] Regina Okner, Abraham Jacob Domb and Daniel Mandler, "Electrochemically deposited poly (ethylene glycol)-based sol–gel thin films on stainless steel stents," *New Journal of Chemistry*, vol. 33, pp. 1596-1604, 2009.
- [21] Hsu-Wei Fang, Kuo-Yen Li, Tai-Lun Su, Thomas Chun-Kuang Yang, "Dip coating assisted polylactic acid deposition on steel surface: Film thickness affected by drag force and gravity," *Materials Letters*, vol. 62, pp. 3739-3741, 2008.
- [22] Sunita Nayak, Tuli Dey, Deboki Naskar, Subhas C. Kundu, "The promotion of osseointegration of titanium surfaces by coating with silk protein sericin," *Biomaterials*, vol. 34, pp. 2855-2864, 2013.

- [23] L. Besra and M. Liu, "A review on fundamentals and applications of electrophoretic deposition (EPD)," *Progress in materials science*, vol. 52, pp. 1-61, 2007.